Public Health Reports

VOLUME 65 AUGUST 18, 1950 NUMBER 33

IN THIS ISSUE

FEDERAL SECURITY AGENCY

PUBLIC HEALTH SERVICE

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Public Health Reports

Vol. 65 • AUGUST 18, 1950 • *No. 33*

Bactericidal Efficiency of Quaternary Ammonium Compounds

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A widespread public health interest in bactericidal agents has developed during the past 50 years. This interest centers particularly on the disinfection of multiple-use eating and drinking utensils in the field of restaurant and hotel sanitation. Official action by the United States Public Health Service in this regard was reported in 1943 in the Ordinance and Code (*1*) recommended for the regulation of eating and drinking establishments. This Public Health Service Ordinance and Code provides for approved bactericidal processes for the disinfection of multiple-service utensils as follows: (a) exposure in water at a temperature of at least 170° F. for at least 2 minutes, or for ½ minute in boiling water; (b) immersion in a lukewarm chlorine bath (hypochlorite) containing at least 50 ppm of uncombined available chlorine for at least 2 minutes, or a concentration of equal bactericidal strength if combined chlorine compounds (chloramines) are used; (c) exposure to steam of at least 170° F. for at least 15 minutes, or at 200° F. for at least 5 minutes, or (d) exposure to a hot air temperature of at least 180° F. for at least 20 minutes. All of these procedures must be carried out under the conditions specified to meet the requirements for each.

Under (b) the Ordinance and Code also provides for the use of chemical bactericidal agents other than chlorine with the following specifications:

1. The health officer concerned must determine that the substituted agent is satisfactory.
2. The concentration of the bactericidal agent must be measurable by a simple and accurate field test.

Suitable compliance with these two requirements is essential if satisfactory results are to be obtained.

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Probably no one will disagree with the statement that exposure to heat, particularly immersion in boiling water, is a more reliable method for disinfecting multiple-service utensils. It is believed that it is the only procedure which will quickly kill pathogenic acid-fast bacteria. However, in many installations, it is not feasible or practicable to provide hot water or heat for the disinfection of utensils. Moreover, in many instances, objection has been made to the use of chlorine on the ground that disagreeable odors are produced which are annoying to employee and customer alike. Under such conditions, evasion of the use, or inadequate use, of chlorine or its compounds may follow. For these and other reasons, the advent of quaternary ammonium compounds for use as bactericidal agents appeared to be a welcome addition to the field. The urge to use quaternary ammonium compounds has increased concurrently with the rapid development in the commercial production, recommendation, and distribution of these compounds.

Fuchs (*2*) in 1947 reviewed the restaurant sanitation program of the Public Health Service and indicated that the Bacteriology Section of the Environmental Health Center at Cincinnati was engaged in a basic study of the bactericidal efficiency of quaternary ammonium compounds (Q.A.C.). The bactericidal action of certain Q.A.C. was reported by Jacobs (*3*) in 1916. Since then a considerable number of articles, pertaining to the Q.A.C., their bactericidal properties, and the factors which affect the latter, have been published. This literature has been adequately reviewed by Rahn and Van Eseltine (*4*). The reader is referred to their article for a more complete discussion of these compounds and their properties. This review definitely indicates the need for more satisfactory laboratory methods for the determination of the bactericidal efficiency of these compounds when they are used in natural waters.

It was natural that the phenol coefficient method of testing disinfectants should be the method of choice in testing the Q.A.C. as they were used first in the medical and surgical field. Such preparations are normally made in distilled water, and the phenol coefficient procedure would produce results of considerable comparative value. In the field of use considered here, however, the operating rather than the comparative efficiency of a disinfectant is of primary concern. When the Q.A.C. are used in natural waters of widely varying composition where inhibiting factors may be involved results obtained with the phenol coefficient test may not constitute an adequate criterion. Klarmann and Wright (*5*, *6*) in studying their own products by the phenol coefficient method found that this procedure tended to show greater bactericidal efficiencies than were observed in practical use. Kolmer and Boerner (*7*) in concluding their discussion of the phenol coefficient test in their text state: "Other groups of disinfect-

ants in common use, for which the phenol coefficient method of testing is not well adapted, are those compounds containing chlorine as the active agent as well as oxidizing agents in general. These are affected so materially by the presence of organic matter that a phenol coefficient statement may grossly misrepresent their value under practical conditions of use, and is very apt to be misleading to the consumer when placed on the label." At best, the phenol coefficient test can give only a relative value of the bactericidal efficiency of a disinfectant under standardized conditions in mixtures of broth and distilled water. It often provides little or no information concerning bactericidal action under conditions of practical use.

The available evidence tends to provide the reasons for the failure of this method of testing the Q.A.C. The bactericidal action of the Q.A.C. is adversely affected by fats and proteins with which they combine, and they are inactivated by soaps, lecithin, and many other substances. This evidence also indicates that if the Q.A.C. are to be used for the disinfection of multiple-service utensils: (1) the utensils must be clean, i.e., free from interfering substances at the time of exposure, and (2) the cumulative effect of small amounts of interfering substances, soaps, etc., carried over into the bactericidal rinses should be considered.

In this study of the bactericidal properties of the Q.A.C., various test methods described in the literature were investigated in addition to the phenol coefficient procedure. A testing method developed at the Environmental Health Center for observations on the bactericidal properties of chlorine (*8*) and chloramines (*9*), which had proved to be very effective, was also included. A modification of this latter procedure was found to be a most effective method for the determination of the bactericidal properties of the Q.A.C. This modification, which will be described in detail, was developed to approximate the actual conditions under which the compounds would be used in practice.

Escherichia coli was selected as the test organism for this study. It would have been desirable to use a variety of test organisms, pathogenic as well as saprophytic, but the volume of work required in investigating the bactericidal efficiency of a very considerable number of Q.A.C. did not permit this. The selection of *E. coli* was based on evidence in the literature which indicated that this organism occupied an intermediate position in its susceptibility to the Q.A.C. It is more resistant than any species of *Streptococcus* or *Staphylococcus* tested, and less resistant than the *Pseudomonas* or *Mycobacterium tuberculosis*. A high resistance of the latter organism would be anticipated as it is very resistant to all processes of chemical disinfection including the use of free chlorine.

In practice, Q.A.C. solutions would be made in the available tap water. Consequently, in exploratory tests a wide variety of

waters were used for the preparation of the solutions of the Q.A.C. under examination. These included distilled water, phosphate buffered distilled water, and tap waters from 10 municipalities. The sources of these tap waters were surface supplies (river and impounded), spring waters, and deep and shallow well supplies. As a result of the information obtained from these data, Cincinnati tap water was selected as representative of an average surface supply and Norwood (Ohio) tap water as an average moderately deep well supply. These two tap waters and buffered distilled water, as a control, were used in the majority of the tests reported at this time.

The standard test portion of water used in this study was 500 ml. in a one-liter flask. This volume provided for (1) rapid and adequate mixing; (2) an ample quantity of sample to permit withdrawal of portions not only for bacterial tests at the various intervals of examination but also for chemical determinations of pH, residual Q.A.C., etc., and (3) protection from temperature changes of the sample, induced by handling, during the period of the test. Temperature changes might alter the course of the bactericidal action. For bacterial examinations only, a lesser sample volume might be used if the necessary precautions were observed. Weber and Black (*11*), after the data for this report had been completed, described such a procedure for bactericidal tests using small amounts of sample.

In bactericidal tests it is essential that the action of the bactericide be stopped exactly at the designated time. In the earlier studies (*8*, *9*) with chlorine and chloramines, this was accomplished by withdrawing portions from the test flasks and discharging these portions at the right time intervals into a measured volume of inhibitor solution which immediately neutralized all chlorine or chloramine present. In preliminary tests on inhibitors of the bactericidal activity of the Q.A.C., it was found that standard Castile soap solutions in low concentrations effectively neutralized their bactericidal action on *E. coli*. It was also found that standard nutrient agar with its organic ingredients would instantaneously neutralize the bactericidal properties of the Q.A.C. for *E. coli* if the dilution of the agar with the solution was kept within reasonable limits. Consequently, in the tests reported at this time the bactericidal action was inhibited by withdrawing 1 and 2 ml. portions of the sample under examination and discharging them into sterile Petri dishes a few seconds before the exposure interval was complete. Then at the correct interval 10 to 15 ml. of standard nutrient agar was poured into the plate directly on the sample portions, rapidly mixed, and congealed. The data obtained indicated that this procedure gave very satisfactory results. If any error was introduced by this method of inhibition, such error would tend to favor the bactericidal potency of the compounds under test.

Methods

Preparation of Glassware

To avoid the possibility of difficulties induced by extraneous materials or organisms, all glassware used in these tests was made clean chemically by treating with acid cleaning solution, rinsing thoroughly with tap and distilled water, draining dry, and sterilizing by exposure to hot air at 180° C. for 2 hours.

Preparation of Bacterial Suspensions

A laboratory strain of *E. coli* (culture No. 198 recently isolated from feces) was used throughout this study. The bacterial suspension was prepared by washing the growth from the entire surface of a standard agar slant which had been inoculated with a young culture and incubated for 20 to 24 hours at 37° C. The growth was washed off aseptically with 2 ml. of sterile phosphate buffered dilution water after which the water containing the culture was returned to the dilution bottle. This bacterial suspension of 99 ml. was shaken vigorously and allowed to stand quiescent for about 10 minutes. An appropriate amount (usually 0.35 ml.) from the supernatant of this suspension was then transferred to a second 99 ml. dilution bottle and again shaken vigorously. By an "appropriate amount" is meant that quantity which, when added to the second dilution bottle, would give a bacterial population in this bottle of about 800,000 per ml. A 1 ml. portion from the second dilution bottle was then added to each test flask containing 500 ml. of the test water, or test water plus bactericidal agent. This method provided a bacterial population of 1,000 to 2,000 per ml. usually about 1,600 per ml. in each flask. The use of such a density, though convenient for the test, might be a less severe procedure for the bactericide than the use of much larger numbers.

Types of Water

As noted, three types of water were selected for use in these studies: (1) distilled water buffered with Clark and Lubs standard phosphate buffer solutions at pH ranges of 7.0 to 9.5; (2) Cincinnati tap water; and (3) Norwood (Ohio) tap water. Tap waters were not sterilized prior to use as this would not occur in practice, and it had been determined in the preliminary work that such sterilization frequently altered the effect of the water on the bactericidal agents subsequently introduced. If the natural tap waters contained any residual chlorine, either free or combined, this was neutralized by the addition of an appropriate amount of sterile N/10 sodium thiosulphate solution. Difficulties incident to the presence of bacteria in the unsterilized tap waters were not encountered as these waters were relatively free of bacteria.

Preparation of Q.A.C. Solutions

Commercial sanitizers were used as put up by the producer. The amount of Q.A.C. present in the dilutions was calculated in parts per million (ppm) on the basis of the amount stated on the label. Solutions of pure Q.A.C. were prepared in distilled water in concentrations corresponding to those given for commercial sanitizers. In initial tests on the bactericidal efficiency of each preparation, the test concentrations were varied within the concentration range recommended for use by the producer. That is, if the producer specified a concentration of 150 ppm, test portions would be made containing 25, 50, 100, 150, 200, and 300 ppm. Concentrations used in subsequently repeated series with the same product would be governed by the results obtained in the initial exploratory tests.

Determinations of Residual Q.A.C.

No attempt was made to develop a test, or tests, for residual Q.A.C. However, tests for residuals were made using 11 commercial test kits which were available on the market. The results obtained with these test kits will be discussed.

Hydrogen-ion Concentration

The hydrogen-ion concentrations of the waters used and of the mixtures of water, bactericidal agent, and bacterial cells were always determined by electrometric methods. The initial pH of the waters could be determined colorimetrically. However, the Q.A.C. interfered with the indicator dyes to such an extent that colorimetric pH determinations were usually unsatisfactory after any of these compounds had been added to the water.

Bacterial Counts

Quantitative determinations of the number of bacteria per ml. in the control portions and of surviving bacteria in test portions after various periods of exposure were made by agar plate counts following the procedures given in Standard Methods (*10*). The only exception to this procedure was that triplicate plates were planted at each dilution instead of duplicate plates. Colonies on plates were counted, using a Quebec Colony Counter, after 24-hours incubation at 37° C. At the inception of the study and at frequent intervals thereafter plates were incubated for longer periods, 48 to 96 hours, to determine if additional colonies might develop. In no instance was any increase in the number of colonies observed. To establish the identity of surviving organisms, isolations were made on standard lactose broth from plates showing a minimum of growth. All colonies on such plates were picked for identification. The colonies selected for study represented the bacteria which had survived the longest exposure time. Contaminations by air borne bacteria were encountered only

rarely and control tests excluded *E. coli* as a contaminant in all such cases. The sterility of Petri dishes, pipettes, water, and agar used in each series was carefully controlled.

Criterion of Satisfactory Disinfection

E. coli was selected as the test organism for this study for reasons which have been given. For purposes of safety, a 100 percent kill of *E. coli* in 1 minute was set as a tentative standard for safe disinfection. The Ordinance and Code (*1*) provides for an exposure time of 2 minutes when such bactericidal agents are to be used. An exposure period of 2 minutes probably exceeds the time that is ordinarily provided in actual operations, particularly during "rush" hours, when the conditions which prevail in hotels, restaurants, and bars are considered. Consequently, it is believed that the 1 minute exposure, with a 100 percent kill, set as the standard criterion for this study, is not too brief an interval; perhaps a shorter interval of 15 or 30 seconds might be desirable under existing conditions.

Tests

In this study, 570 series of tests have been made in addition to a considerable number of preliminary exploratory experiments. The methods, materials, and equipment described here were used. A "series" consisted of repeated observations on several test portions of water, usually eight. In a series, 500 ml. portions of tap waters (or other water used) were added to each of eight sterile 1-liter Erlenmeyer flasks. Flask 1, subjected to the same handling and treatment as the remaining flasks, was used as a temperature control. Flask 2, also a control, contained test water only, received no Q.A.C. solution, and was used to determine the bacterial behavior in the water under test. To the third and all succeeding flasks, increasing amounts (expressed in ppm of active agent) of the Q.A.C. under test were added. One ml. portions of bacterial suspension were then introduced into all flasks at appropriate intervals. "Appropriate interval" means that additions of bacteria to each succeeding flask were made with such intervening time periods that subsequent examinations of the various test portions at the indicated times could be made without conflict. Coincident with the addition of the bacteria, vigorous mixing was started and continued for 30 seconds. Routine bacterial plate counts were made after 1-, 2-, and 5-minute periods of exposure, with occasional examinations after 10, 30, and 60 minutes. The hydrogen-ion concentration of each water was determined for each series and for each test portion at the end of each series. Tests for residual Q.A.C. in which available commercial test kits were used were made frequently on each test portion at the end of a run.

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Results

In the 570 series of tests, 40 commercial sanitizers containing Q.A.C. as the bactericidal agent were included. Eleven chemically different types of Q.A.C. were represented as active agents in this group. Several of these Q.A.C. in pure form (filler-free) were included in this study. The 570 series required the examination of approximately 20,000 bacteriological samples in addition to the examinations of the preliminary tests and the identification of surviving bacteria. It is not practicable to present all of the data in detail and no attempt is made to do so. Presentation of the data is confined to averaged results showing the reproducible nature, or reliability, of the testing procedure used and to summaries illustrating the factors which were found to affect the bactericidal efficiency of these compounds. Also, the effectiveness of available test procedures for the determination of residual Q.A.C. is discussed.

Duplicability of Procedure

The all-important characteristic of any test procedure is that it be reliable. Repeated examinations of the same sample should produce results which are in reasonable agreement. In conducting tests in which many variable factors are involved, it is essential to standardize or eliminate variables in so far as possible in order to determine the reliability of the testing procedure.

Table 1. *Results obtained in repeated tests of 2 compounds in Cincinnati tap water, using the described procedures*

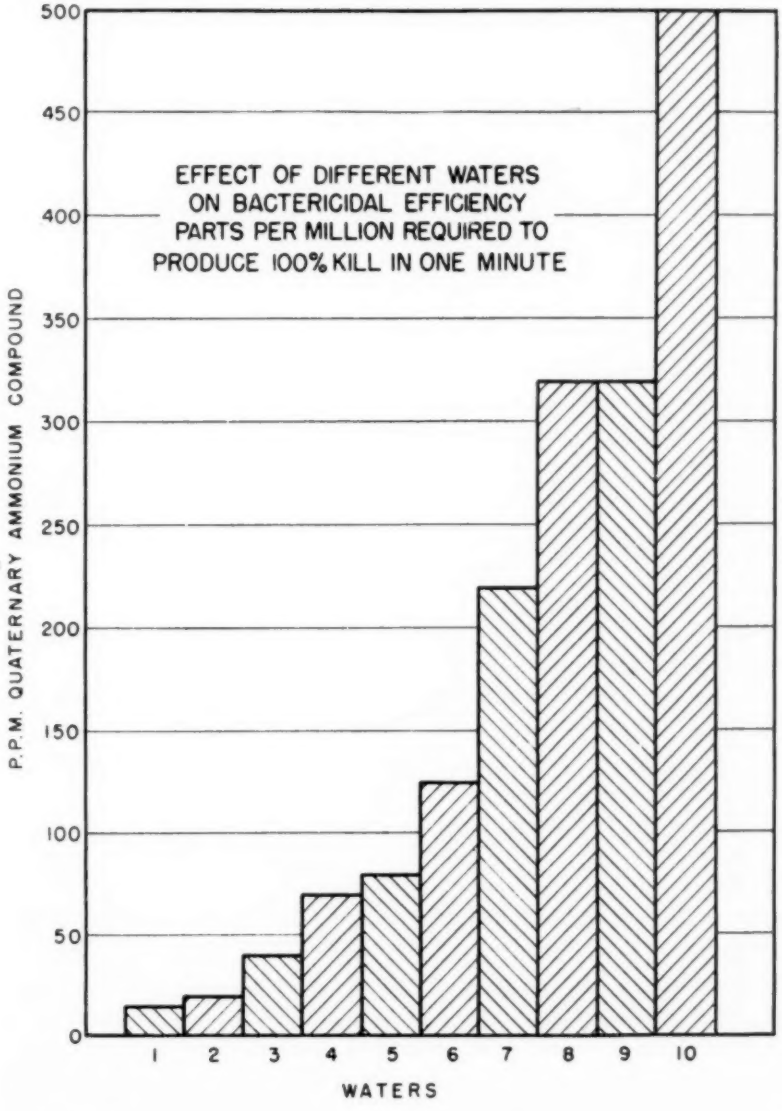
Compound tested	Test number	Parts per million of active agent required to obtain a 100-percent kill		
		1 minute	*2 minutes*	*5 minutes*
I	1	150	100	50
I	2	150	100	50
I	3	150	100	50
I	4	100	75	50
P	1	125	100	75
P	2	125	100	50
P	3	125	100	50

In table 1 the results of repeated examinations on the bactericidal efficiency of two widely used quaternary ammonium compounds are presented. In these tests aliquots of the same water were used for each experiment, and the pH and the temperature were kept constant. It may be noted that for compound I the maximum variations observed were 33, 25 and 0 percent, respectively, for the 1-, 2-, and 5-minute periods of exposure; for compound P the maximum variation was also 33 percent. Such variations are well within the limits of the probable error of such bacterial determinations, and they were much

less than the variations with other test procedures investigated. Moreover, these variations are very much less than the safety factors (twofold to fiftyfold) usually required for the use of such disinfecting agents. It is believed that this test procedure gives a reliable measure of the bactericidal efficiency of these compounds.

Effect of Water on Bactericidal Efficiency

In selecting standard test waters for this study, 10 waters were investigated. One of the more widely used quaternary ammonium compounds was the bactericidal agent employed. The results are shown in the chart.



Waters 1, 2, and 3 were distilled water buffered at pH 8.0, 7.0, and 6.6, respectively, with Clark and Lubs phosphate buffers. The other waters were: 4, Wyoming (Ohio) drilled well supply; 5, Harrison (Ohio) drilled well supply; 6, Cincinnati, treated Ohio river water; 7, Norwood (Ohio) well supply; 8, Lebanon (Ohio) drilled well supply; 9, Lawrenceburg (Indiana) tubular well supply; and 10, Cincinnati, tap to which a trace of sodium borate was added.

The amount of active agent required to produce a 100 percent kill of *E. coli* in 1 minute in these waters varied from 15 to 500 ppm. Five waters required less than, and five more than, 100 ppm. The average ppm of Q.A.C. required was 170. Waters 6 and 7, Cincinnati tap water (a representative river supply), and Norwood tap water (a representative well water) which were selected as the test waters for this study, required, respectively, 125 and 220 ppm of this active agent. As the requirement for these waters was approximately 30 percent less than, and 30 percent more than, respectively, the average amount required for all waters investigated, it was considered that they would be appropriate for use as standard test waters.

In this connection it should be noted that synthetic waters simulating these natural waters in their chemical constituents were investigated for use as possible test waters. No combinations of chemical constituents in distilled water were found which would reproduce consistently the results obtained with natural waters. Moreover, any modification of the natural water, such as boiling, autoclaving, filtration, etc., invariably, materially altered the degree to which this water affected the bactericidal efficiency of the Q.A.C. under test. This effect was observed even though no change in pH occurred and no visible precipitation was noted in the waters under treatment. This leads to the conclusion that the bactericidal efficiency of these compounds is markedly affected by constituents of natural waters other than the *chemical constituents usually determined.* Consequently, any opinion regarding the factors affecting bactericidal efficiency of these compounds based on such partial information would be unsound and probably misleading.

Relative Efficiency of Various Q.A.C.

In table 2 results obtained with 15 Q.A.C. sanitizers in synthetic, Cincinnati and Norwood tap waters are presented. Of the 15 representative compounds, 4 were pure, unadulterated with either filler or diluent, and 1, designated T, was a combined detergent-sanitizer, made up from the combination of a detergent and a sanitizing agent, each of which was better than average for its purpose when tested independently. The results obtained with T are included in the

Table 2. *Effect of water on the bactericidal efficiency of quaternary ammonium compounds dissolved therein*

Active agent* designation	Results obtained at room temperature in waters indicated									Ppm. recommended for use by producer
	Buffered distilled			Cincinnati tap			Norwood tap			
	Ppm. required to produce a 100 percent kill in following minutes									
	1	2	5	1	2	5	1	2	5	
1	100	75	50	300	300	200	500	400	300	.....
2	75	50	25	300	125	100	300	225	150	.....
5	40	30	20	150	80	40	200	150	100	150
A	40	.....	20	175	.....	100	300	200	150	264
D	50	.....	.....	200	200	100	300	160	120	158
I	30	20	10	100	70	50	250	175	125	234
L	75	50	25	600	300	200	800	400	.....	188
M	30	.....	10	200	150	100	300	225	200	.....
P	40	30	10	125	100	100	220	120	80	150
S	30	20	10	90	80	40	300	200	.....	192
T	30	30	5	500	400	400	15,000+	15,000+	15,000+	.....
U	10	.....	2.5	100	80	50	140	120	40	.....
W	40	20	.....	100	50	40	150	125	100	.....
Z	100	70	30	400	200	100	450	350	225	.....
A-1	75	25	.....	120	110	80	325	200	120	.....
Average	71	38	18	231	160	133	1,302	1,203	1,285	.....
Average omitting T	52	39	19	211	142	93	324	217	142	.....

*The active agents were: for 1, A and D, para-diisobutylphenoxyethoxyethyldimethylbenzyl ammonium chloride; for 2, diisobutylcresoxyethoxyethyldimethylbenzyl ammonium chloride; for 5, P, S, T, U, and W, alkyldimethylbenzyl ammonium chloride; for L, N (acylcolamineformylmethyl) pyridinium chloride; and for M, para-tertiaryoctylphenoxyethoxyethyldimethylbenzyl ammonium chloride. The active agents for I, Z, and A-1 were not definitely determined.

table to illustrate the effect which was encountered consistently with detergent-sanitizer combinations. Two sets of averages were prepared for the results in this table. One excluded the results obtained with T, as they were so abnormal that the averages which included them were not representative.

The marked effect of the diluting water on the bactericidal efficiency was observed with every compound. In general, the pattern of the interference was the same in all cases regardless of whether the comparisons were made for 1-, 2-, or 5-minute intervals of exposure. For practical application, the 1-minute exposure results are of greatest significance. The average parts per million of active agent of these 14 compounds required to produce a 100 percent kill of *E. coli* in 1 minute in synthetic, Cincinnati and Norwood tap waters, respectively, were 52, 211, and 324, approximately a ratio of 1:4:6. A review of the data in the table shows that these ratios for the individual compounds are not too divergent, varying from a minimum of approximately 1:2:4 with A-1 to a maximum of 1:10:14 with U. In all cases the influence of the water on the bactericidal effectiveness of the compounds was quite marked.

In the case of product T referred to above, which was a combination of a sanitizer and a detergent, the ppm required for a 1-minute kill in the three waters were 30, 500, and more than 15,000, respectively; a ratio of approximately 1:17:500. It should be noted here that the

active agent in T (with detergent added) was the same as the active agent in U (without detergent). It is evident from the data that the detergent interfered markedly with the bactericidal efficiency of the active agent, and that this interference was not the same for different waters. That is, the average ratio of ppm of active agent required for Cincinnati tap to the ppm for Norwood tap is less than 1:2 for all products other than T, while for T this ratio was 1:30 or more (500:more than 15,000).

In the last column of table 2 there is recorded the ppm of active agent recommended for use by the producers of some of the products tested. It will be observed from the data in the table that in no instance was this recommended dosage sufficient to produce a 100 percent kill of the bacteria under test in Norwood tap water in 1 minute; also that in only 4 out 7 cases was the recommended dosage sufficient to produce this effect in Cincinnati tap water. This failure to recommend the use of an adequate amount of their products, however, should not be construed as an indication of unreliability or of a lack of integrity on the part of the producers of these products, for as has been indicated, these producers in making, or having made, tests on the bactericidal efficiency of their products, undoubtedly relied on the results of tests which were made either in distilled or in buffered distilled waters (phenol coefficient tests).

The bactericidal efficiency obtained in using buffered distilled water yielded results as observed in table 2, which made the producers' recommendations provide safety factors of from twofold to eightfold. For this reason it is believed that it was undoubtedly the intent of the producers to recommend for their respective products dosages which would provide for a 100-percent kill of vegetative bacteria in 1 minute with a liberal factor of safety. The interference produced by natural waters in which the products were used was responsible for the failure in the effectiveness of the dosages recommended.

Rapidity of Interference Reaction

Observations were made to determine whether the reduction in bactericidal efficiency produced by the diluting water was an instantaneous or a progressive reaction. To do this, master portions of diluted Q.A.C. were prepared. An initial test of the bactericidal properties was made as quickly as possible after the dilution was completed (usually within 1 to 2 minutes); a second portion was tested after the stock dilution had stood at room temperature for 4 hours, and a third portion after 24 hours. All tests were made by the standard procedure. Three representative Q.A.C. were used in these tests with both Cincinnati and Norwood tap waters. All tests were repeated at least once; a total of 66 observations were made in this series. In no test was there any result indicating that a significant reduction

in bactericidal efficiency had occurred after the initial examination; slight variations in the ppm required were observed, but these were all well within the limits established in table 1. This leads to the conclusion that in general the compounds are stable in these waters after the initial reaction reducing the bactericidal efficiency has taken place, and that this initial reaction occurs instantaneously or at least during the first minute of contact. These observations are supported by the fact that, among large numbers of bottles of commercial products (usually 10 percent concentrations of Q.A.C.) standing on laboratory shelves for a year or longer, in only one instance was deterioration of the product determined. In this one case active bacterial growth occurred in a stock commercial bottle.

Effect of Temperature

In investigating the influence of temperature on the bactericidal properties of the Q.A.C. a temperature range of from 12° to 46° C. was used. This range was selected as representing the extremes (based on sensory reactions) which probably would be encountered in hand-dishwashing procedures. For purposes of illustration the results obtained have been grouped in ranges of 12° to 17° C., 18° to 26° C., 33° to 39° C., and 40° to 46° C. These results are presented in table 3.

Table 3. *Effect of temperature variations on bactericidal properties*

Q.A.C. compound	Ppm of Q.A.C. which will produce a 100 percent kill in 1, 2, and 5 minutes in Cincinnati tap water in temperature ranges of:											
	12°–17° C.			18°–26° C.			33°–39° C.			40°–46° C.		
	1	2	5	1	2	5	1	2	5	1	2	5
A				175		160	100	80	60	60		40
D	225	200	150	200	200	100	100		75			
L				100		50	80		20	30		
M				200	150		150		100			
P	200	150	100	125	100	100	70	40	30	30	20	
S	200	150	100	90	80	40	60		40	30	20	
U	100	80	40	100	75	50						
Average	181	145	98	141	121	83	93		54	38		

Q.A.C. compound	Ppm of Q.A.C. which will produce a 100 percent kill in 1, 2, and 5 minutes in Norwood tap water in temperature ranges of:											
	12°–17° C.			18°–26° C.			33°–39° C.			40°–46° C.		
	1	2	5	1	2	5	1	2	5	1	2	5
A				300	200	150	200	150		160	120	80
D	500	500	350	300	160	120	250	250	150			
L				250	175	125	100	50	50	50		25
M				300	225	150	250	200	150			
P	500	400	200	220	120	80	125	100	100	60	40	20
S	400	300	200	500	200		75	50	25	55		
U	600	500	200	140	120	40						
Average	500	425	238	287	171	111	167	133	95	81		42

In this section of the study, no effort was made to examine statistically the temperature coefficient relations as the other variables and uncontrolled interfering factors did not justify such critical study. However, the recorded results show the marked influence of temperature on the bactericidal efficiency of these compounds. In general the concentration required to obtain a 100 percent kill in 1 minute at the temperature ranges of 12°–17° C., 18°–26° C., and 33°–39° C. were respectively five, three and two times that required in the range of 40°–46° C. This temperature effect was consistent in all tests, and for all periods of exposure although, as would be anticipated, the differential tended to decrease as the period of exposure was increased.

Influence of Hydrogen-ion Concentration

In exploring the effect of hydrogen-ion concentration on the bactericidal activity of these compounds, more than 130 tests were made using 33 different products, in pH ranges of 6.5 to 9.5. These limiting ranges were used as it was not likely that waters of a pH value below 6.5 would be encountered and at pH ranges of 9.5 and above the hydrogen-ion concentration of the water would be a factor in the bactericidal process. In all tests, the results showed that the bactericidal efficiency was affected by changes in the hydrogen-ion concentration. However, with approximately 50 percent of the products examined, their bactericidal activity was enhanced by increases in pH, and with the other half a lowering of the pH increased the bactericidal action, apparently to about the same degree. Consequently, no general statement can be made regarding the influence of pH on the bactericidal action of the Q.A.C. except that they are all affected by the hydrogen-ion concentration of the suspending menstrum. The direction and extent of the effect must be determined for the particular product in use.

Substances Inhibiting Bactericidal Efficiency

Extensive exploratory tests were made to determine the agents which were responsible for the lowering of the bactericidal efficiency of these compounds. Tests were made with inorganic and organic compounds, and various detergents; and detailed studies, with solutions of Castile soap. With some of the Q.A.C., the presence of small amounts of phosphate completely inhibited their bactericidal action. Calcium and magnesium salts markedly restricted the activity of all of the compounds tested. At one time during the course of the study, it was believed that the degree of interference of a water could be correlated with the concentration of the salts present which are normally responsible for the hardness of waters. However, as the study progressed, it was observed that *"hardness" compounds were*

only one of the many factors which impaired efficiency. The removal of dissolved gases by aeration reduced the interference of some waters. Boiling or sterilizing by autoclaving two of the natural tap waters under test markedly reduced their interfering action although no visible precipitation or clouding occurred which would indicate the removal of some substances from solution. It is believed that the efficiency of the Q.A.C. as bactericidal agents is reduced by a variety of substances and compounds, many of which have not been determined or the extent of their interference measured.

In the tests with Castile soap seven of the more generally used Q.A.C. were studied. The amount of soap required to destroy the effective action of the concentration of Q.A.C. recommended for use by the producer varied from 1 to 60 ppm; one required only 1 ppm, and two each of the other six required 10, 20, and 60 ppm, respectively, to produce the same effect. This indicates the necessity of adequately rinsing a utensil before it is exposed in the Q.A.C. sanitizer bath. In this connection, the possibility of the production of interfering soaps at the site through the interaction of the alkaline Q.A.C. concerned and fat or oil films left on the surface of utensils should not be overlooked.

The magnitude of the effect of interfering substances on the bactericidal action of the Q.A.C. varies under the conditions of use and especially with the nature of the water in which they are used. This is of the greatest importance when detergent-sanitizer combined preparations are considered. Even though it involves repetition this point is emphasized by referring again to the average results obtained with preparations U and T as recorded in table 2. Preparation T differed from U only in that a good detergent had been incorporated in T. Basing the comparison on the 1-minute kill, the results obtained in pure water (which would be used ordinarily by a producer in testing his product) show that the addition of the detergent reduced the bactericidal effectiveness of the sanitizer threefold. However, the results also show that in two tap waters the addition of the detergent reduced the bactericidal activity fivefold and more than a hundredfold, respectively.

Tests for Residuals

The Public Health Service Ordinance and Code (*1*) provides for the use of chemical bactericidal agents subject to certain conditions. One of these conditions is that the residual concentration of the active bactericidal agent must be measurable by a simple and accurate field test. The primary importance of this requirement is illustrated excellently in the development of the practice of chlorination as a bactericidal process in the water purification field. With the introduction of the use of chlorine as a bactericidal agent in water purification and the ensuing spectacular results, it was believed generally

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that the addition of a certain fixed amount of chlorine (a recommended dosage) would provide for adequate disinfection of all waters. Several years were spent in practical search for this ideal chlorine dosage. It was found that it was not the initial amount of chlorine added which determined the effectiveness of the disinfection process, but more probably the amount of chlorine remaining in the water (the residual) after a certain period of contact. Tests were devised for the determination of residual chlorine and various periods of exposure were investigated. Still success was not attained, for it was found that residuals effective for one water supply were ineffective for others. There was no consistency in the results obtained. Then it was learned that when chlorine was added to water, addition products were formed which varied with the chemical composition of the water to which the chlorine was added. It was also learned that (1) these addition products were not as effective bactericidal agents as chlorine, in some instances relatively ineffective, and (2) these addition products were measured as chlorine by the current tests for residuals. New residual tests were developed which would differentitate between uncombined chlorine and its addition products—tests which measured residual chlorine in terms of its bactericidal efficiency. At last success was attained in the control of the disinfection of water with chlorine. The experience with disinfection by chlorine should serve as a guide for the control of the use of other chemical bactericidal agents.

In the present study tests for residual bactericide were made on nine representative Q.A.C. with 11 commercial test kits which were available for use. Test kits which failed to measure with a fair degree of accuracy the residuals of the product for which they were designed when the Q.A.C. was dissolved in buffer distilled water were judged to be useless. Five of the kits used fell within this category. The results obtained with the remaining six are presented in table 4.

Table 4. ***Relation of bactericidal activity to residual Q.A.C. as determined by field test kits***

Q.A.C. compound	Ppm of Q.A.C. required for a 100 percent kill in 1 minute		Ppm of residual Q.A.C. determined by test kit		Test kit used	Ratio ppm required for 1 minute kills—Cincinnati tap: pure water
	Pure water	Cincinnati tap water	Pure water	Cincinnati tap water		
L	75	600	100−	600	a	8 : 1
P	20	200	20±	200−	b	10 : 1
S	30	200	30±	200	c	7 : 1
U	0.5	100	Too low to test	100	b	200 : 1
T	5	3,000	Too low to test	3,000±	b	600 : 1
D	<50	225	50	200+	d	5 : 1
C	60	220	50+	200+	e	4 : 1
M	30	200	30±	200	d	7 : 1
O-I	50	6,400	50	6,400±	f	128 : 1

It may be noted that in all instances, in both buffered distilled water (pure water) and in Cincinnati tap, all six test kits measured with a high degree of accuracy residuals in terms of the amount of Q.A.C. added initially. However, in natural waters these readings did not bear any relation to the amount of active bactericidal residual present, indicating residuals which were in error by from fourfold to six hundred fold. For instance, in line one of table 4 test kit "a" indicated the presence of 600 ppm of residual Q.A.C. (the amount which had been added at the start), whereas the bacteriological evidence, repeatedly confirmed, indicated that only 75 ppm of active bactericidal agent were present. The remaining 525 ppm of residual Q.A.C. measured by this test kit represented an altered compound which was no longer actively bactericidal. As shown in the table, the magnitude of these errors is so great that the results obtained with these kits with natural waters would be valueless and very misleading. Studies looking toward the development of such residual tests are being made by Weber (*11*).

Tests which measure residuals in terms of their active bactericidal content are a primary essential for the economic and safe use of any chemical method of disinfection. In using disinfecting procedures in the absence of satisfactory residual tests, the only safe alternative is to make bacteriological examinations with the product under consideration in the water to be used and under the conditions in which it will be used.

Summary

The general characteristics of the Quaternary Ammonium Compounds and various test methods described in the literature for the determination of their bactericidal efficiency are reviewed. A detailed procedure is described for the determination of the bactericidal efficiency, under operating conditions, of Q.A.C and other chemical agents proposed for use as bactericides. Using this procedure with *Escherichia coli* as the test organism, the bactericidal efficiency of 11 quaternary ammonium compounds which are used as the active agents in 40 commercial sanitziers has been determined. Tests for residual active agents were also made.

The results obtained show that:

1. The bactericidal efficiency is affected markedly by the nature of the water in which the compound is used. The concentration of active agent required to produce a 100-percent kill in 1 minute varies from 15 to 500 ppm.

2. The interference with bactericidal efficiency induced by different waters occurs almost instantaneously and does not increase with time of exposure.

3. Within the range, 12° to 46° C., which might be used, temperature has a marked influence on the bactericidal efficiency of these compounds, the higher the temperature the more effective the toxic action.

4. The toxic action is affected by changes in the hydrogen-ion concentration of the solutions, but the direction of change varies with the compound—the decreases enhance the potency of some compounds and reduce that of others.

5. The bactericidal efficiency is reduced by various substances and compounds in addition to the "hardness" salts. Small amounts of soap or other detergent almost invariably reduces the action markedly. With some waters the removal of dissolved gases by aeration or boiling reduces the interference with the bactericidal activity.

6. Test procedures available for the determination of residual compounds in terms of active bactericidal agent yield very unreliable results in natural waters.

7. In the absence of any satisfactory residual test for determining the amount of effective bactericidal agent present, it is essential to control the action by bacteriological examinations with the product under the conditions in which it is used.

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Haplomycosis in Montana Rabbits, Rodents, and Carnivores

By William L. Jellison*

Haplomycosis is the name proposed by Emmons (*1*) for a disease of animals characterized by the presence in the lungs of the fungus *Haplosporangium parvum*. This fungus was first cultured during a survey of mycotic infections of rodents in southern Arizona by Emmons and Ashburn (*2*). Of the 303 rodents examined and cultured in their survey, 25 were found infected with *Coccidioides immitis* and 101 with *H. parvum*. Nine rodents were infected with both fungi.

A wide range of rodent hosts in nature is indicated by the variety of animals found infected in Arizona which included 23 of 124 *Perognathus*, 3 of 29 *Dipodomys*, 5 of 10 *Citellus*, 1 of 27 *Onychomys*, and 2 of 113 *Peromyscus*.[1]

The presence of *Haplosporangium parvum* in native rodents in Alberta, Canada, was noted by Dowding (*3*, *4*) soon after the Emmons and Ashburn survey. Dowding found large fungus cells in the lungs of 14 animals and established the fungus in culture from 8 of these. Infected animals included 13 white-footed deer mice, *Peromyscus maniculatus borealis*, and one red squirrel, *Tamiasciurus hudsonicus baileyi*. In a later publication, Dowding (*5*) reported that the infection had probably been found in muskrats in British Columbia by Ian McTaggert Cowan of the University of British Columbia, but she did not state on what evidence the diagnosis had been based. Numerous infected muskrats have been found in western Montana as reported later in this paper.

What appears to be the first observation on haplomycosis, although not identified at the time, was made by Dr. Arnold B. Erickson of the University of Minnesota and reported in 1949 (*6*). The lungs of a beaver collected in Aitkin County, Minnesota, March 31, 1941, were observed to contain an abundance of small discrete white nodules. The writer has examined sections of this material and agrees that the organism is *Haplosporangium* sp., although presence of infection in rodents in that area has not been confirmed by culture.

In 1944 the writer found and later recorded (*7*) the presence of

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[1] The species studied in this survey by Emmons were: *Peromyscus eremicus, Perognathus baileyi, P. penicillatus, P. intermedius, Dipodomys merriami,* and *Citellus harrisi.* One or more animals of each species was found infected according to Emmons (personal communication, Feb. 8, 1950).

some unidentified bodies, presumably parasites, in the lungs of a rock rabbit, *Ochotona princeps*, in Ravalli County, Montana. The fungoid nature of these bodies was suspected but culture was not attempted. A similarly infected rock rabbit was collected nearby in Granite County, Montana, August 19, 1947, by Major Robert Traub, parasitologist of the Army Medical School, and the writer. Culture was attempted from this animal but was not successful.

The fungoid nature of these bodies remained unconfirmed until July 1949, when a cottontail rabbit, *Sylvilagus nuttallii*, shot in Ravalli County, Montana, was found on examination to have small scattered white nodules in the lung tissue similar to those found in the rock rabbits examined previously. Individual cysts were teased out, washed repeatedly in sterile saline, and planted on tubes of Littman's medium (*8*). Mycelial growth developed in about one week. Transfers have been identified as *Haplosporangium* sp. and the identification confirmed by Dr. Emmons. Typical infections in experimental animals have been produced by this culture.

Although not specifically recognized at the time, the first infected animal found in western Montana was a large female skunk, *Mephitis hudsonica*, trapped March 9, 1944, at Post Creek, Lake County, Montana, by Delbert Palmer of Charlo, and autopsied by the writer. Blood samples were being collected from skunks for serological studies. The lungs of this animal showed numerous discrete white spots and so were preserved in formalin for histological study. When sections were made and examined in 1949 by Dr. J. K. Frenkel, pathologist at the Rocky Mountain Laboratory, the white spots were identified as nodules containing fungus cells of *Haplosporangium* sp. This diagnosis has been further confirmed by establishing cultures from several other skunks in Lake County during November 1949.

Lung sections from a wood rat, *Neotoma fuscipes*, collected near Hastings Natural History Reservation, California, were sent to the writer by Dr. Jean M. Linsdale. These sections contained numerous bodies typical of the cells of *Haplosporangium* sp. The presence of this fungus in *Neotoma* and other small mammals on the Hastings Reservation has since been confirmed by culture (Emmons, unpublished data).

There remains some question as to the specific identity of the fungus found in Canadian and Montana mammals. The species found in Arizona was named *H. parvum* by Emmons and Ashburn (*2*) and was in part characterized by the spherical fungus cells of about 14μ in diameter found in the lung tissue. In experimentally infected animals the fungus cells reached a diameter of 40μ. On the basis of mycelial growth and conidiospore formation, Dowding (*3*) identified cultures from rodents in Alberta as *H. parvum*. However, she observed that the fungus cells in lung tissue reached a size

of 270μ. Measurements of similar cells from *Ochotona* in Ravalli County were 360μ to 390μ. More detailed studies on the cultural characters of numerous strains of this fungus and of their infectivity for experimental animals are being made by Dr. Emmons and by the writer.

Since the culture and identification of *Haplosporangium* sp. from the cottontail collected July 22, 1949, a rather extensive survey has been made of the native animals in western Montana and northern Idaho by autopsy, direct examination, and culture for the presence of this fungus. Numerous species of both rodents and carnivores have been found infected.

Survey Studies

In September 1949 Dr. C. W. Emmons, mycologist from the National Institutes of Health, visited the Rocky Mountain Laboratory to confer with the writer on this problem and to examine native animals in the laboratory and in the field. One trip was made by Dr. Emmons, William Fullerton, and the writer to Blue Nose Peak, elevation 8,887 ft., on the Montana-Idaho Divide, 60 miles south of Hamilton, Mont., where rock rabbits, *Ochotona princeps*, were known to be quite abundant. Autopsies and cultures were made in the field on rock rabbits and other freshly shot or trapped animals. A few of the mice trapped were held in below-freezing temperature for later examination. Other captured animals were examined at the laboratory.

This work resulted in establishment of cultures of *Haplosporangium* sp. from the following hosts: one female wood rat, *Neotoma cinerea*, from near Boulder Creek, West Fork of the Bitter Root River, Ravalli County, trapped by Harley Sargent, laboratory aide; one rock rabbit, *Ochotona princeps*, shot on Blue Nose Peak, Montana-Idaho Divide; one white-footed deer mouse, *Peromyscus maniculatus*, trapped near Horse Creek Pass, Lemhi County, Idaho.

In July 1949, a trip was made to Lake County, Mont., with Harley Sargent, to collect and examine additional skunks for infection with *Haplosporangium* sp. Through the cooperation of Delbert and Louis Palmer, of Charlo, eight skunks were trapped or shot, including seven young of the year and one adult animal. Typical fungus cells were found in the lungs of the adult animal. Cultures were made on all eight skunks; no isolations were obtained.

A third trip was made to Lake County in November 1949 to work with Delbert and Louis Palmer who were trapping in the vicinity of Charlo. At this time 23 skunks were examined and cultured. Cultures typical of *Haplosporangium* sp. were established from three skunks, *Mephitis hudsonica*. Very heavy concentrations of fungi were

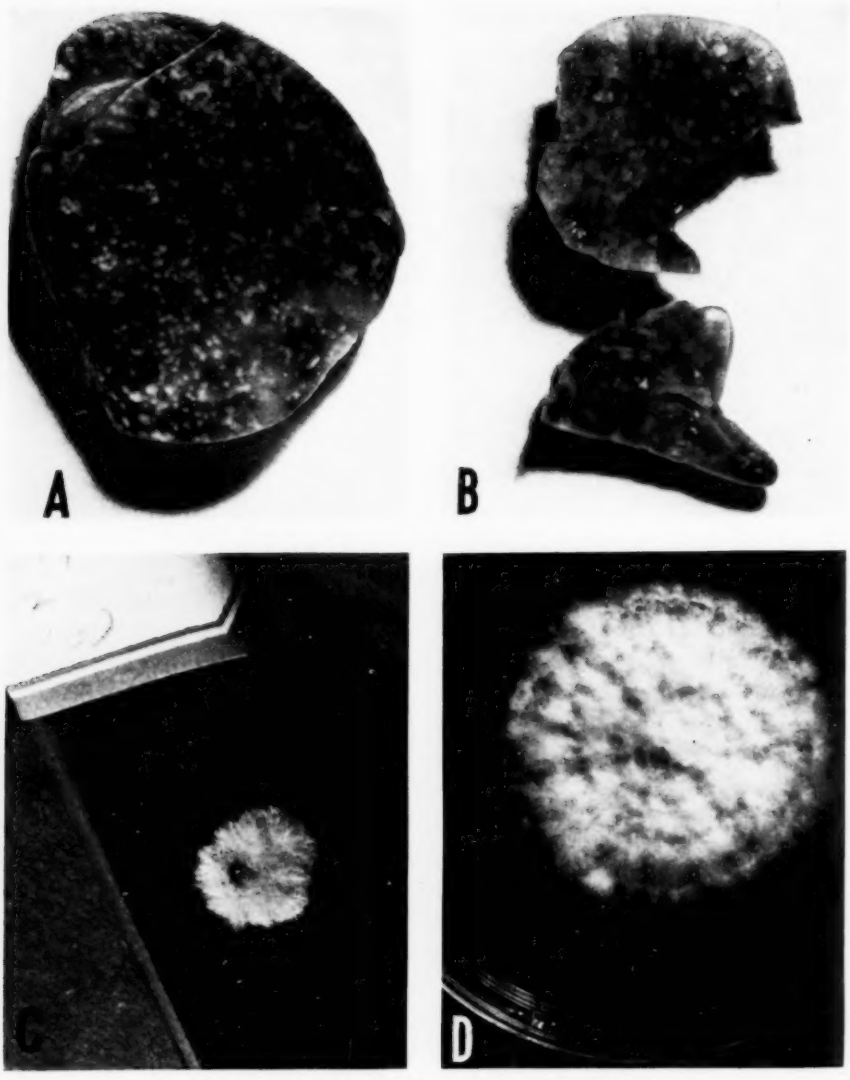

Figure 1. *Haplosporangium* sp. *in vivo* and *in vitro*.

A. Portion of lung of skunk with natural infection, Lake County, Mont.
B. Portion of lung of muskrat with natural infection, Lake County, Mont.
C. Test tube culture from skunk, Lake County, Mont.
D. Petri dish culture from deer mouse, Lemhi County, Idaho.

found in the lungs of several animals. Two of these infected skunks came from a den of seven found under a chicken house at the Roberts farm south of Charlo.

The Palmer brothers were also trapping weasels and mink and, though muskrat season was not open, a few of these animals taken inadvertently in mink sets were brought in for examination. No cul-

tures were established and no fungi were found in any of the mink examined from Lake County. Cultures typical of *Haplosporangium* sp. were established from three of the weasels, two *Mustela frenata* and one *M. erminea*, collected in Lake County.

Muskrats, *Ondatra zibethica*, trapped on the Wall farm, 1½ miles north of Charlo, showed such extremely heavy infestations that considerable areas of the lungs appeared consolidated. Cultures of *Haplosporangium* sp. were established from three of four muskrats from this farm and from one muskrat on the Morris farm near Charlo. This would tend to confirm the suspected findings of *Haplosporangium* sp. in muskrats in British Columbia as reported by Dowding (*5*).

Arrangements were made with the Palmer family to save in cold storage the lungs of a series of muskrats to be trapped when the regular season opened. On December 6, frozen lungs were obtained from 126 muskrats which were trapped in the general vicinity of Charlo. No attempt was made to culture these lungs, but they were examined microscopically before and after partial digestion in 2 percent NaOH solution. Typical fungus cells were found in 23 sets of lungs, or 18 percent of the animals. Infestations varied from single cells to almost complete consolidation of the lungs by masses of fungi and their surrounding tissue nodules.

In the course of other survey studies, cells typical of *Haplosporangium* have been found in the following hosts: an adult female, *Peromyscus maniculatus*, Ravalli County, October 26, 1949, trapped by Lawrence Humble (a culture was established from a single cell found in this animal); a weasel, *Mustela frenata*, Ravalli County, November 3, 1949, shot by Martin Shoffner (a single cell found); a weasel, *M. frenata*, Ravalli County, July 22, 1949, shot by the writer (this animal had an extremely heavy infection); a pine squirrel, *Tamiasciurus hudsonicus*, shot in Skalkaho Canyon, Ravalli County, November 17, 1949, by Dr. Robert Philip and William Fullerton; a pine marten, *Martes americana*, trapped near Holland Lake, Missoula County, Mont., December 1949 by Dr. Philip Wright, Professor of Zoology, University of Montana (single cell found in lungs); three of four beavers, *Castor canadensis*, trapped in Ravalli County, December 1949, by M. J. Watt, Deputy Game Warden; a mink, *Mustela vison*, trapped in Ravalli County, March 8, 1950. These and other collections are shown in the table.

Summary

Haplomycosis is a pulmonary disease of mammals caused by infection with one or more species of the fungus *Haplosporangium*. It was first found in ground squirrels, mice, and kangaroo rats in a semi-desert area in Arizona. Mice and a tree squirrel in Alberta, Canada,

Rodents, rabbits, and carnivores found infected with Haplosporangium *sp.*

Animal No.	Host	Location	Date	Fungus cells in lungs	Cultures established
20777	Skunk	Lake Co., Mont	Mar. 9, 1944	+	
21285	Rock rabbit	Ravalli Co., Mont	July 24, 1944	+	
21840	do	Granite Co., Mont	Aug. 19, 1947	+	
26528	Skunk	Lake Co., Mont	July 14, 1949	+	
26756	Wood rat	Ravalli Co., Mont	Sept. 1, 1949		+
26761	Rock rabbit	do	Sept. 9, 1949		+
26850	Weasel	do	July 22, 1949	+	+
26852	Cottontail	do	do	+	+
27097	White-footed mouse	Lemhi Co., Idaho	Sept. 3, 1949	+	+
27110	do	Ravalli Co., Mont	Oct. 26, 1949	+	+
27113	Weasel	do	Nov. 3, 1949	+	
27123	Skunk	Lake Co., Mont	Nov. 16, 1949	+	+
27124	do	do	do	+	+
27128	do	do	do	+	+
27130	Muskrat	do	Nov. 15, 1949	+	+
27135	Pine squirrel	Ravalli Co., Mont	Nov. 17, 1949	+	
27188	Weasel	Lake Co., Mont	Nov. 1949	+	+
27189	do	do	Nov. 1949	+	+
27190	Muskrat (23 of 126 positive)	do	Dec. 1949	+	
27199	Beaver	Ravalli Co., Mont	Dec. 1949	+	
27210	Pine marten	Missoula Co., Mont	Dec. 1949	+	
27242	Muskrat	Lake Co., Mont	Nov. 18, 1949	+	+
27255	do	do	Nov. 20, 1949	+	+
27258	Weasel	do	Nov. 1949		+
27261	Muskrat	do	Nov. 21, 1949	+	+
27274	Weasel	Ravalli Co., Mont	Jan. 23, 1950		+
27308	Cottontail	do	do	+	
27320	Beaver	do	Feb. 1950	+	
27321	do	do	Feb. 1950	+	
27330	Muskrat	Lake Co., Mont	Feb. 12, 1949	+	
27342	Weasel	Ravalli Co., Mont	Feb. 14, 1950	+	
27347	White-footed mouse	do	Feb. 13, 1950	+	
27357	Muskrat	do	Mar. 2, 1950	+	
27358	Muskrat (2 of 6 positive)	do	do	+	
27382	Muskrat (2 of 4 positive)	do	Mar. 4, 1950	+	+
27387	Mink	do	Mar. 8, 1950	+	
27440	Muskrat	Powell Co., Mont	Mar. 20, 1950	+	+

and a beaver in Minnesota have been reported infected. This fungus is recorded here for the following hosts from western Montana: beaver, muskrat, pine squirrel, and white-footed mouse of the order Rodentia; rock rabbits and cottontails of the order Lagomorpha; mink, pine marten, skunk, and weasel of the order Carnivora. The known mammalian hosts of *Haplosporangium* spp. have a wide geographical, ecological, and zoological distribution.

ACKNOWLEDGMENT

This study was carried out as a cooperative project with Dr. C. W. Emmons, Principal Mycologist, Laboratory of Infectious Diseases, National Institutes of Health, Bethesda, Md. Besides the accessions noted, Biological Aides Lawrence M. Humble and William T. Smith collected many of the animals examined. Most of the laboratory examinations were made by Miss Roma Fullberg, Biological Aide. Special thanks are due the E. M. Palmer family at Charlo, Montana, who secured animals for study in Lake County and provided field laboratory facilities at their home on several occasions. The weasels were determined by Dr. Philip Wright, Professor of Zoology, University of Montana.

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Communities Awarded Milk Sanitation Ratings of 90 Percent or More, July 1948–June 1950 [1]

This is the semiannual revision of the list of Public Health Service milk ordinance communities which were reported by State milk-sanitation authorities during the 2-year period July 1, 1948, to June 30, 1950, as having a market milk rating of at least 90 percent. The inclusion of a community in this list means that, if pasteurized milk is sold in the community, it is of such a degree of excellence that the weighted average of the percentages of compliance with the various items of sanitation required by the Public Health Service Milk Ordinance for grade A pasteurized milk is 90 percent or more, and that, similarly, if raw milk is sold in the community, it so nearly meets the standards that the weighted average of the percentages of compliance with the various items of sanitation required for grade A raw milk is 90 percent or more.

These ratings are not a complete measure of safety, but represent the degree of compliance with the grade A standards. High-grade pasteurized milk is safer than high-grade raw milk because of the added protection of pasteurization. Safety estimates should take into account the percentage of milk pasteurized, which is given in the table. To obtain this added protection, those who are dependent on raw milk can pasteurize the milk at home by the use of an approved home pasteurizer or by either of the following methods: (1) after the water in the bottom of a double boiler has been brought to a vigorous boil, place the inner container with milk in the outer container, cover it, and continue to apply the same heat for 10 minutes; or (2) heat the milk in an open saucepan over a hot flame to 165° F., stirring constantly, then immediately place the vessel in cold water and continue stirring until cool, changing the water when it warms up; however, if a dependable thermometer is not available, bring the milk to a boil instead. Method 1 produces a cooked flavor, while method 2 is not quite as safe as method 1.

The milk ordinance recommended by the Public Health Service is now in effect State-wide in 13 States, as well as in 360 counties and 1,464 municipalities located in 39 States. It has been adopted as a regulation by 34 States and Territories.

The primary reason for publishing the rating lists is to encourage these communities to attain and maintain a high level of excellence in the enforcement of the ordinance. No comparison with communities operating under other milk ordinances is intended or implied. Some

[1] From Division of Sanitation, Milk and Food Branch.

communities which have high-grade milk supplies are not included because arrangements have not been made for the determination of their ratings by the State milk sanitation authority. In other cases the ratings which have been submitted are now more than 2 years old and have therefore lapsed. In still other communities with high-grade milk supplies there seems, in the opinion of the community, to be no local necessity nor desire for rating or inclusion in the list.

The rules under which a community is included in this list are as follows:

1. All ratings must be determined by the State milk-sanitation authority in accordance with the Public Health Service rating method.[2] based upon the grade A pasteurized milk and the grade A raw milk requirements of the Public Health Service Milk Ordinance and Code. A recent departure from the method described consists of computing the pasteurized milk rating by weighting the plant rating twice as much as the rating of the raw milk for pasteurization.

2. No community will be included in the list unless both its pasteurized milk and its raw milk ratings are 90 percent or more. Communities in which only raw milk is sold will be included if the raw milk rating is 90 percent or more.

3. The rating used will be the latest rating submitted to the Public Health Service, but no rating will be used which is more than 2 years old. In order to promote continuous rigid enforcement rather than occasional "clean-up campaigns" it is suggested that when the rating of a community on the list falls below 90 percent no resurvey be made for at least 6 months, which will result in removal from the next semiannual list.

4. The Public Health Service will make occasional check surveys of cities for which ratings of 90 percent or more have been reported by the State. If such check rating is less than 90 percent but not less than 85, the city will be removed from the 90-percent list after 6 months unless a resurvey submitted by the State during this probationary interim shows a rating of 90 percent or more. If, however, such check rating is less than 85 percent, the city will be removed from the list immediately. If the check rating is 90 percent or more, the city will be retained on the list for a period of 2 years from the date of the check survey unless a subsequent rating submitted during this period warrants its removal.

Communities which are now on the list should not permit their ratings to lapse since ratings more than 2 years old cannot be used.

State milk-sanitation authorities who are not now equipped to determine municipal ratings are urged, in fairness to their communities, to equip themselves as soon as possible. The personnel required is small; in most States one milk specialist is sufficient for this work.

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[2] Pub. Health Report 53:1386 (1938). Reprint No. 1970.

Communities Awarded Milk Sanitation Ratings of 90 Percent or More, July 1948–June 1950

Community	Percent of milk pasteurized	Date of rating
ALL MARKET MILK PASTEURIZED		
ALABAMA		
Auburn	100	Sept. 29, 1949
Birmingham and Jefferson County	100	Nov. 17, 1949
Montgomery	100	May. 11, 1950
COLORADO		
Colorado Springs	100	Nov. 1949
Grand Junction	100	Mar. 29, 1950
FLORIDA		
Panama City	100	Sept. 18, 1948
GEORGIA		
Columbus	100	Oct. 27, 1949
Cordele	100	Sept. 8, 1949
Quitman	100	Aug. 25, 1949
West Point	100	Mar. 29, 1949
IDAHO		
Bonners Ferry	100	Mar. 14, 1949
Caldwell	100	Apr. 14, 1949
Idaho Falls	100	Aug. 24, 1949
Preston	100	Nov. 16, 1948
Sandpoint	100	May 14, 1949
ILLINOIS		
Champaign-Urbana	100	Aug. 18, 1948
Chicago	100	Oct. 28, 1949
Decatur	100	Apr. 27, 1950
East Moline	100	May 18, 1950
Elgin	100	Dec. 8, 1949
Glencoe	100	Nov. 7, 1949
Highland Park	100	Nov. 7, 1949
Kenilworth	100	Nov. 7, 1949
Lake Bluff	100	Nov. 7, 1949
Lake Forest	100	Nov. 7, 1949
Moline	100	May 18, 1950
Northfield	100	Nov. 7, 1949
Oak Park	100	Sept. 1949
Peoria	100	Apr. 15, 1950
Rock Island	100	May 10, 1950
Silvis	100	May 18, 1950
Skokie	100	Nov. 7, 1949
Waukegan	100	Nov. 2, 1949
Winnetka	100	Nov. 7, 1949
INDIANA		
Anderson	100	Dec. 19, 1949
Hope	100	1950
Indianapolis	100	July 1948
South Bend	100	Nov. 1948
KANSAS		
Dodge City	100	May 24, 1950
KENTUCKY		
Hopkinsville	100	Mar. 1950
Owensboro	100	Apr. 8, 1949
Paducah	100	May 5, 1950
MISSOURI		
Columbia	100	Dec. 1949
NORTH CAROLINA		
Charlotte	100	Feb. 23, 1950
Cumberland County	100	Feb. 10, 1950
Mars Hill	100	Dec. 7, 1949
Transylvania County	100	Jan. 16, 1949
OKLAHOMA		
Cushing	100	Feb. 10, 1950
TENNESSEE		
Athens	100	June 14, 1950
Bristol	100	Nov. 4, 1949
Chattanooga	100	Oct. 26, 1949
Columbia	100	Apr. 20, 1950
Erwin	100	Feb. 17, 1949
Fayetteville	100	May 10, 1949
Franklin	100	May 5, 1950
Greenville	100	Oct. 7, 1949
Kingsport	100	Sept. 23, 1949
Knoxville	100	Sept. 23, 1949
Lewisburg	100	Apr. 17, 1950
Maryville-Alcoa	100	Aug. 31, 1948
Morristown	100	Oct. 13, 1949
Shelbyville	100	June 13, 1949
TEXAS		
Galveston	100	Apr. 18, 1949
Gladewater	100	July 25, 1949
Harlingen	100	Mar. 20, 1950
Houston	100	Dec. 3, 1948
Kilgore	100	July 25, 1949
Lufkin	100	Apr. 12, 1949
San Antonio	100	Mar. 11, 1950
Texarkana	100	Mar. 30, 1949
Texas City	100	Apr. 25, 1949
Tyler	100	Mar. 2, 1950
Weslaco	100	Apr. 5, 1950
UTAH		
Ogden	100	June 1, 1949
Provo	100	Apr. 29, 1949
Salt Lake City	100	May 27, 1949
VIRGINIA		
Boydton	100	Apr. 4, 1950
Bristol	100	Nov. 4, 1949
Lawrenceville	100	Apr. 6, 1950
Pulaski	100	June 1950
Radford	100	June 1950
Richmond	100	May 1950
Suffolk	100	May 24, 1950
Waynesboro	100	May 19, 1949

Communities Awarded Milk Sanitation Ratings of 90 Percent or More, July 1948–June 1950—Continued

Community	Percent of milk pasteurized	Date of rating
BOTH RAW AND PASTEURIZED MARKET MILK		
GEORGIA		
La Grange	76.2	Mar. 29, 1949
Macon	97.1	Sept. 13, 1949
Thomaston	79.7	May 24, 1950
Thomasville	81.5	July 28, 1948
IDAHO		
Boise	99.3	Apr. 30, 1949
Payette	72	Apr. 14, 1949
Weiser	92.1	Apr. 13, 1949
KENTUCKY		
Lexington and Fayette County	96	June 23, 1950
NORTH CAROLINA		
Alexander County	73.5	Mar. 31, 1950
Avery County	73.5	July 12, 1949
Buncombe County	95	June 10, 1949
Cabarrus County	73.4	Jan. 20, 1950
Henderson County	86	Feb. 2, 1950
Wilkes County	89.7	Jan. 25, 1950
OKLAHOMA		
Ada	84	June 24, 1949
Holdenville	89	Mar. 28, 1950
Lawton	96	Feb. 20, 1950
Shawnee	96	May 25, 1949
OKLAHOMA—Con.		
Stillwater	96	July 7, 1949
Sulphur	98	Sept. 6, 1949
OREGON		
Portland	99.2	July 24, 1949
TENNESSEE		
Jackson	95.8	Mar. 30, 1950
McMinnville	95.1	May 25, 1950
Murfreesboro	98	July 27, 1949
Pulaski	91.6	May 6, 1949
TEXAS		
Brenham	92	Apr. 15, 1950
Brownsville	84.8	Mar. 20, 1950
Bryan	98.8	Feb. 12, 1949
Corsicana	99.6	Jan. 31, 1950
Edinburg	85.9	Apr. 5, 1950
Fort Worth	99.95	Feb. 4, 1950
Longview	99	July 27, 1949
Lubbock	98.2	July 15, 1949
Palestine	79.8	Apr. 28, 1949
Paris	91.8	Dec. 13, 1949
VIRGINIA		
Emporia	34	Apr. 7, 1950

NOTE: In these communities the pasteurized market milk shows a 90 percent or more compliance with the grade A pasteurized milk requirements and the raw market milk shows a 90 percent or more compliance with the grade A raw milk requirement of the Public Health Service Milk Ordinance and Code.

Note particularly the percentage of milk pasteurized in the various communities listed. This percentage is an important factor to consider in estimating the safety of a city's milk supply. All milk should be pasteurized or boiled, either commercially or at home, before it is consumed. See text for home method.

Incidence of Disease

No health department, State or local can effectively prevent or control disease without knowledge of when, where, and under what conditions cases are occurring

UNITED STATES

Reports From States For Week Ending July 29, 1950

For the current week in the Nation, new cases of acute poliomyelitis numbered 970, an increase over the preceding week's total for the tenth consecutive week. However, the total for the current week is less than the 1,956 cases reported for the corresponding week last year. The cumulative total number of reported cases for the current "disease" year was 4,985, less than the 7,364 cases reported for the corresponding period of last (1949–50) year, the highest year on record. The current cumulative total is also less than the 5,443 cases reported for the corresponding period in the 1948–49 "disease" year. The disease year for acute poliomyelitis begins with the twelfth week of the calendar year. The cumulative total number reported for the

Comparative Data for Cases of Specified Reportable Diseases: United States

[Numbers after diseases are International List numbers, 1948 revision]

Disease	Total for week ended		5-year median 1945–49	Seasonal low week	Cumulative total since seasonal low week		5-year median 1944–45 through 1948–49	Cumulative total for calendar year		5-year median 1945–49
	July 29, 1950	July 30, 1949			1949–50	1948–49		1950	1949	
Anthrax (062)			(1)	(1)	(1)	(1)	(1)	27	33	(1)
Diphtheria (055)	53	96	140	27th	176	280	391	3,304	4,048	6,688
Acute infectious encephalitis (082)	31	12	12	(1)	(1)	(1)	(1)	452	311	275
Influenza (480–483)	514	398	571	30th	276,789	112,137	182,373	246,259	75,867	138,815
Measles (085)	2,502	1,977	2,058	35th	301,584	636,241	578,358	282,454	583,848	543,412
Meningococcal meningitis (057.0)	50	47	54	37th	3,413	3,037	3,299	2,500	2,193	2,327
Pneumonia (490–493)	697	745		(1)	(1)	(1)		58,469	53,240	
Acute poliomyelitis (080)	970	1,956	910	11th	[2] 4,985	7,364	3,699	[2] 6,119	8,279	4,166
Rocky Mountain spotted fever (104)	41	40	40	(1)	(1)	(1)	(1)	277	347	302
Scarlet fever (050)	279	207	466	32d	[2] 56,095	79,724	87,883	[2] 39,656	57,180	61,197
Smallpox (084)			1	35th	43	49	196	23	39	142
Tularemia (059)	28	24	24	(1)	(1)	(1)	(1)	596	727	599
Typhoid and paratyphoid fever (040, 041) [3]	104	294	146	11th	1,290	1,493	1,493	1,800	1,981	1,981
Whooping cough (056)	2,630	1,592	2,428	39th	100,736	43,443	85,841	79,200	33,410	57,712

[1] Not computed.
[2] Mississippi: Deductions—week ended July 15, poliomyelitis, 2 cases; scarlet fever, 2 cases.
[3] Including cases reported as salmonellosis.

current calendar year is 6,119 compared with 8,279 reported for the corresponding period in 1949.

For the current week, all geographic divisions except the East South Central and Mountain showed increases over the preceding week. These increases ranged from 2 cases (163 to 165) in the West South Central Division to 43 cases (93 to 136) in the Middle Atlantic. The East South Central Division decreased by 27 cases (105 to 78), and the Mountain States decreased by 6 cases (22 to 16). Texas reported the largest number of cases (107); New York, the second highest (90); and Virginia, the third (82).

The 514 cases of influenza reported for the current week in the Nation ended the seasonal year for this disease. The cumulative total beginning with the 31st week of 1949 was 276,789 and is the median for the past 5 "seasonal" years. The highest total number of cases reported during this period was 552,445 in the 1945–46 season, and the lowest was 112,137 reported during the 1948–49 season.

Total reported cases of pneumonia numbered 82,939 for the "disease" year ended with this report. For the corresponding period in the previous year 73,404 cases of pneumonia were reported.

Thirty-one cases of infectious encephalitis were reported during the current week compared with 12 for the corresponding week last year. Of this total, 15 cases were reported in California and 5 in Texas. The cumulative total number of acute infectious encephalitis cases reported for the calendar year is 452 compared with the corresponding total of 311 for 1949 and the 5-year median of 275.

Reported cases of whooping cough continued to increase over the preceding week. No smallpox was reported in the United States.

Deaths During Week Ended July 29, 1950

	Week ended July 29, 1950	*Corresponding week, 1949*
Data for 94 large cities of the United States:		
Total deaths	8, 284	8, 931
Median for 3 prior years	8, 504	
Total deaths, first 30 weeks of year	282, 485	281, 618
Deaths under 1 year of age	588	690
Median for 3 prior years	690	
Deaths under 1 year of age, first 30 weeks of year	18, 659	19, 432
Data from industrial insurance companies:		
Policies in force	69, 691, 785	70, 309, 604
Number of death claims	13, 170	12. 364
Death claims per 1,000 policies in force, annual rate	9. 9	9. 2
Death claims per 1,000 policies, first 30 weeks of year, annual rate	9. 6	9. 4

Reported Cases of Selected Communicable Diseases: United States, Week Ended July 29, 1950

[Numbers under diseases are International List numbers, 1948 revision]

Area	Diphtheria (055)	Encephalitis, infectious (082)	Influenza (480–483)	Measles (085)	Meningitis, meningococcal (057.0)	Pneumonia (490–493)	Poliomyelitis (080)
United States	**53**	**31**	**514**	**2,502**	**50**	**697**	**970**
New England		**1**	**1**	**186**		**21**	**39**
Maine				2		4	5
New Hampshire							1
Vermont				3			
Massachusetts		1		143			7
Rhode Island			1	1		3	3
Connecticut				37		14	14
Middle Atlantic	**12**	**3**	**3**	**740**	**8**	**120**	**136**
New York	6	3	[1] 3	275	3	80	90
New Jersey	5			274	2	23	26
Pennsylvania	1			191	3	17	20
East North Central	**5**	**2**	**15**	**750**	**6**	**101**	**140**
Ohio			1	125	2	14	26
Indiana	1			34		3	5
Illinois			1	188	1	38	52
Michigan	2	1	1	119	3	42	44
Wisconsin	2	1	12	284		4	13
West North Central	**1**	**2**	**1**	**109**	**4**	**31**	**113**
Minnesota			1	20	1	9	8
Iowa				5	2		62
Missouri				60	1	6	12
North Dakota				2		14	1
South Dakota		2					2
Nebraska	1			14		1	14
Kansas				8		1	14
South Atlantic	**16**		**98**	**107**	**10**	**85**	**197**
Delaware				2			4
Maryland	8		3	17		32	9
District of Columbia			1	1		11	9
Virginia			76	34	5	21	82
West Virginia			6	11		2	13
North Carolina	3			21	3		30
South Carolina	3		11	2	2	5	34
Georgia	2		1	1		3	8
Florida				18		11	8
East South Central	**4**	**1**	**11**	**66**	**7**	**48**	**78**
Kentucky				30	2	12	34
Tennessee	1		7	9	2		13
Alabama	2	1	2	18	2	25	18
Mississippi	1		2	9	1	11	13
West South Central	**14**	**6**	**340**	**125**	**10**	**202**	**165**
Arkansas			15	10	1	13	23
Louisiana	2			5	1	21	10
Oklahoma	1	1	12	5	1	11	25
Texas	11	5	313	105	7	157	107
Mountain		1	37	184	2	56	16
Montana			11	4		1	2
Idaho			4	14	1		3
Wyoming				2		5	1
Colorado			5	90		10	6
New Mexico			3	16		22	2
Arizona		1	14	8	1	15	2
Utah				50		2	
Nevada						1	
Pacific	**1**	**15**	**8**	**235**	**3**	**33**	**95**
Washington				18	1		20
Oregon	1		6	13		3	8
California		15	2	204	2	30	67
Alaska							
Hawaii					1		1

[1] New York City only.

Reported Cases of Selected Communicable Diseases: United States, Week Ended July 29, 1950—Continued

[Numbers under diseases are International List numbers, 1948 revision]

Area	Rocky Mountain spotted fever (104)	Scarlet fever (050)	Smallpox (084)	Tularemia (059)	Typhoid and paratyphoid fever (040,041)[1]	Whooping cough (056)	Rabies in animals
United States	**41**	**279**		**28**	**104**	**2,630**	**116**
New England		**31**			**1**	**295**	
Maine		1				53	
New Hampshire						4	
Vermont						36	
Massachusetts		27			1	122	
Rhode Island		1				36	
Connecticut		2				44	
Middle Atlantic	**3**	**42**			**11**	**316**	**36**
New York		[2] 28			5	141	32
New Jersey	3	5			1	102	
Pennsylvania		11			5	73	4
East North Central	**4**	**87**			**5**	**552**	**10**
Ohio		21				98	3
Indiana	3	3			1	21	
Illinois	1	13			2	93	2
Michigan		39			1	205	5
Wisconsin		11			1	135	
West North Central		**14**			**2**	**269**	**4**
Minnesota		4				61	
Iowa		1				99	4
Missouri		3			1	62	
North Dakota						4	
South Dakota						7	
Nebraska		2				8	
Kansas		4			1	28	
South Atlantic	**22**	**30**		**4**	**28**	**329**	**18**
Delaware		1				3	
Maryland	6	2				45	
District of Columbia		1				3	
Virginia	7	9		2	4	72	3
West Virginia					4	47	1
North Carolina	8	9		1	6	106	
South Carolina					5	9	6
Georgia	1	8		1	8	11	8
Florida					1	33	
East South Central	**6**	**20**		**2**	**19**	**122**	**27**
Kentucky		10			7	60	16
Tennessee	4	7			7	37	5
Alabama	2	1			2	19	6
Mississippi		2		2	3	6	
West South Central	**3**	**16**		**18**	**22**	**397**	**19**
Arkansas	2	2		13	3	15	2
Louisiana				1	6	7	
Oklahoma	1	1		1	2	20	2
Texas		13		3	11	355	15
Mountain	**3**	**5**		**4**	**6**	**160**	
Montana	1	2		3		20	
Idaho	1	1			3	18	
Wyoming	1					3	
Colorado		1			1	21	
New Mexico		1			1	26	
Arizona					1	57	
Utah				1		15	
Nevada							
Pacific		**34**			**10**	**190**	**2**
Washington		8			2	49	
Oregon		3			1	43	
California		23			7	98	2
Alaska						9	
Hawaii						3	

[1] Including cases reported as salmonellosis.

[2] Including cases reported as streptococcal sore throat.

FOREIGN REPORTS

REPORTS OF CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER RECEIVED DURING THE CURRENT WEEK

The following reports include only items of unusual incidence or of special interest and the occurrence of these diseases, except yellow fever, in localities which had not recently reported cases. All reports of yellow fever are published currently. A table showing the accumulated figures for these diseases for the year to date is published in the PUBLIC HEALTH REPORTS for the last Friday in each month.

Cholera

Burma. During the week ended July 8, 1950, 66 cases of cholera, with 53 deaths, were reported in Burma. One case was reported in the port of Toungoo during the week ended July 1.

India. According to information dated July 21, 1950, an outbreak of cholera is occurring in Bombay. As of that date 171 new cases, 9 deaths, had been reported since July 16.

Indochina (French). During the week ended July 1, 1950, one fatal case of cholera was reported in the Thudaumot rural area, Viet Nam.

Plague

Belgian Congo. Plague has been reported in Stanleyville Province, as follows: Week ended July 8, 1950, one fatal case at Antonio, northeast of Blukwa; week ended July 15, one fatal case at Govi, north of Blukwa.

Indochina (French). On July 13, 1950, one fatal case of plague was reported at Govap, Cochinchina.

Smallpox

Argentina. During the month of May 1950, 164 cases of smallpox were reported in Argentina, including 52 cases in Buenos Aires Province, 14 in Corrientes Province, 11 in San Luis Province, 40 in Rio Negro Territory, and 22 in Neuquen Territory.

Cameroon (British). During the week ended June 10, 1950, 30 cases of smallpox were reported.

Indonesia. Information dated July 17, 1950, states that the epidemic of smallpox which began in Surabaya, Java, the last week in February 1950, when 21 cases were reported for that week, showed no sign of abatement as of June 29. Through the week ended June 24, 1,751 cases, with 509 deaths, had been reported. No figures for the week ended July 1 have been received, but 184 cases were reported for the week ended July 8. In Pontianak, Borneo, 16 cases, 5 deaths, were reported for the week ended June 17, and 19 cases, 9 deaths, for the week ended June 24.

Typhus Fever

Jamaica. During the week ended July 15, 1950, 3 cases of typhus fever (murine type) were reported in Kingston.

Spain. During the week ended June 17, 1950, nine cases of typhus fever, one fatal, were reported in Malaga Province.

Yellow Fever

Cameroon (French). The fatal suspected case of yellow fever reported on July 6, 1950, in Foumban Region (see PUBLIC HEALTH REPORTS, August 4, 1950, p. 1001) was not confirmed.

State and Territorial Health Officers 1950 Meeting

The 49th Annual Conference of the Surgeon General of the Public Health Service and the Chief of the Children's Bureau with the State and Territorial Health Officers, State Mental Health Authorities, and State Hospital Survey and Construction Authorities will be held in Washington, D. C., Monday through Friday, October 23 to 27, 1950. This year the State and Provincial Health Authorities will participate in the program, which includes a two-day scientific session to be held Tuesday and Wednesday, October 24 and 25, at the National Institutes of Health, Bethesda, Md.

Plans and arrangements for this conference, which is held in conjunction with the annual meeting of the Association of State and Territorial Health Officers, are being coordinated by the Division of State Grants in the Bureau of State Services.

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The printing of this publication has been approved by the Director of the Bureau of the Budget (August 10, 1949).

The PUBLIC HEALTH REPORTS, first published in 1878 under authority of an act of Congress of April 29 of that year, is issued weekly by the Public Health Service through the Division of Public Health Methods, pursuant to the following authority of law: United States Code, title 42, sections 241, 245, 247; title 44, section 220.

It contains (1) current information regarding the incidence and geographic distribution of communicable diseases in the United States, insofar as data are obtainable, and of cholera, plague, smallpox, typhus fever, yellow fever, and other important communicable diseases throughout the world; (2) articles relating to the cause, prevention, and control of disease; (3) other pertinent information regarding sanitation and the conservation of the public health.

The PUBLIC HEALTH REPORTS is published primarily for distribution, in accordance with the law, to health officers, members of boards or departments of health, and other persons directly or indirectly engaged in public health work. Articles of special interest are issued as reprints or as supplements, in which forms they are made available for more economical and general distribution.

Requests for and communications regarding the PUBLIC HEALTH REPORTS, reprints, or supplements should be addressed to the Surgeon General, Public Health Service, Washington 25, D. C. Subscribers should remit direct to the Superintendent of Documents, Washington 25, D. C.

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UNITED STATES GOVERNMENT PRINTING OFFICE, WASHINGTON, D. C. : 1950

For sale by the Superintendent of Documents, United States Government Printing Office, Washington 25, D. C. Price 10 cents. Subscription price $4.00 a year.